In the Claims

Claim 25 (currently amended) A method of operating absorbance-based chemical sensors to achieve calibration-free measurements, the method comprising the steps of:

a) establishing wavelength accuracy to within about 2-3 nanometers;

b) eliminating stray light at all wavelengths to about less than 0.1% incident light;

c) preparing an analyte-selective reagent at a concentration;

d) equilibrating the analyte-selective reagent to an analyte;

e) taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a first wavelength ($I_{\lambda 1}$) with a reagent-based optical chemical sensor, wherein the sensor has been modified to allow the renewal of an analyte-selective reagent, wherein the first wavelength corresponds to an un-reacted form of the analyte-selective reagent, and taking an intensity reading of the equilibrated analyte-selective reagent and analyte at a second wavelength ($I_{\lambda 2}$), wherein the second wavelength corresponds to a reacted form of the analyte-selective reagent;

f) replacing the equilibrated analyte-selective reagent and analyte with a spectrophotometric blank solution;

g) taking an intensity reading of the blank solution at the first wavelength ($I_{\lambda 10}$), and taking an intensity reading of the blank solution at the second wavelength ($I_{\lambda 20}$);

h) calculating an absorbance ratio using the equation $A_R=A_{\lambda 1}/A_{\lambda 2}$, where A_R is the absorbance ratio, $A_{\lambda 1}$ is absorbance at the first wavelength and $A_{\lambda 2}$ is absorbance at the second wavelength and, wherein $A_{\lambda 1}$ and $A_{\lambda 2}$ are determined by

$$A = -\log (I_\lambda / I_{\lambda 0})$$

~~$A_\lambda = \log I_\lambda$~~

~~$I_{\lambda 0}$~~; and

i) calculating the sensor response with the molar absorptivities (ϵ) of the reacted (a) form of the analyte-selective reagent and the un-reacted form (b) of the analyte-selective reagent using the equation

$$R = -\log\left(\frac{A_R - \epsilon_{\lambda 1a}/\epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b}/\epsilon_{\lambda 2a} - A_R\epsilon_{\lambda 2b}/\epsilon_{\lambda 2a}}\right) = +pK_a - pH,$$

$$R = -\log\left(\frac{A_R - \epsilon_{\lambda 1a}/\epsilon_{\lambda 2a}}{\epsilon_{\lambda 1b}/\epsilon_{\lambda 2a} - A_R\epsilon_{\lambda 2b}/\epsilon_{\lambda 2a}}\right) = +pK_a - pH,$$

wherein when the analyte-selective reagent is prepared accurately and reproducibly at the concentration sensor readings between sensors are calibration-free.